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PATENT CHEM&amp;ENU

## WHAT IS CLAIMED IS:

1. A chemical analysis method for determining chemically related differences between subject biological material and control biological material, comprising the steps of:

(a) providing:

- 5 (i) control biological material,  
(ii) subject biological material, and  
(iii) a fluid extractant containing an aqueous isopropanol- KOH mixture or aqueous isopropanol;

10 (b) contacting the control biological material with the fluid extractant to produce at least one fluid extract of the control biological material;

(c) contacting the subject biological material with the fluid extractant to produce at least one fluid extract of the subject biological material;

15 (d) chromatographing the fluid extract of the control biological material to produce a control chromatogram of the fluid extract of the control biological material; and

(e) chromatographing the fluid extract of the subject biological material to produce a subject chromatogram of the fluid extract of the subject biological material; and

(f) determining the differences between the subject chromatogram of step (e) and the control chromatogram of step (d) to identify at least one outlier peak.

20 2. The method according to Claim 1, further comprising, after step (f), the step:  
(g) determining the chemical identity of the outlier peak(s).

3. The method according to Claim 1 wherein:

- 25 (A) step (c) is performed prior to or simultaneously with step (b); or  
(B) step (e) is performed prior to or simultaneously with step (d); or  
(C) both (A) and (B).

30 4. The method according to Claim 1 wherein steps (b) and (d) are both performed prior to step (c).

5. The method according to Claim 1, wherein liquid chromatography is used in steps (d) and (e). ?

35 6. The method according to Claim 5, wherein gas chromatography/mass spectroscopy is used in step (g). 2?

40 7. The method according to Claim 1, wherein in step (f), the differences between the subject chromatogram and control chromatogram are determined by a computerized data processing technique.

45 8. The method according to Claim 1 further comprising a step of fractionating said fluid extracts of the subject and control biological material(s) to produce a series of fractions for each of said fluid extracts, prior to said steps (d) and (e) of chromatographing said fluid extracts, wherein said steps (d) and (e) of chromatographing said fluid extracts comprise chromatographing said fractions.

9. A method for determining chemically related differences between subject biological material and control biological material, comprising the steps of:

(A) providing:

- 50 (1) at least one sample of at least one subject biological material, and

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- (2) at least one sample of at least one control biological material;
- (B) optionally reserving at least one portion of each said sample of biological material, thereby producing a reserved portion of, and leaving a remainder sample of, each said sample of biological material;
- 5 (C) extracting each said sample or remainder sample of biological material with a fluid extractant, said extractant comprising
- (1) an aqueous isopropanol- KOH mixture, or
- (2) aqueous isopropanol,
- 10 thereby producing an original extract of each said sample or remainder sample of biological material;
- (D) optionally splitting each said original extract into a first portion and a second portion;
- (E) preparing a set of reconstituted first fractions and a set of first aqueous phases by
- (1) adding to, and mixing with, each said original extract, or with each said first portion thereof, a non-polar organic solvent,
- 15 (2) allowing said organic solvent and said original extract to coalesce to form a first organic phase and a first aqueous phase,
- (3) separating said first organic phase from said first aqueous phase, each said separated first organic phase then being a first fraction, and each said separated first aqueous phase being a member in said set of first aqueous phases,
- 20 (4) evaporating to dryness each said first fraction, thereby producing a first fraction residue, and
- (5) reconstituting said first fraction by adding to, and mixing with, said first fraction residue, a non-polar organic solvent to produce a reconstituted first fraction, each of said reconstituted first fractions being a member in said set of reconstituted first fractions;
- 25 (F) optionally splitting each said first aqueous phase into a first portion and a second portion;
- (G) preparing at least one set of esterified second fractions and, optionally, a set of second aqueous phases, by either
- (1) performing
- 30 (a) a fractionation technique comprising the steps of
- (i) acidifying each said first aqueous phase to a pH below pH6, to form an acidified first aqueous phase,
- (ii) adding to, and mixing with, each said acidified first aqueous phase, a non-polar organic solvent,
- 35 (iii) allowing said organic solvent and said acidified first aqueous phase to coalesce to form a second organic phase and a second aqueous phase,
- (iv) separating said second organic phase from said second aqueous phase, each said separated second organic phase then being a second fraction, and each said separated second aqueous phase being a member in said set of second aqueous phases; and
- 40 (b) a reconstitution technique comprising the steps of

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- (i) evaporating to dryness each said second fraction, thereby producing a second fraction residue, and
- (ii) reconstituting said second fraction by adding to, and mixing with, said second fraction residue, a non-polar organic solvent to produce a reconstituted second fraction; and
- (c) a methylation of said reconstituted second fraction,
- thereby producing an esterified second fraction, each said esterified second fraction being a member in said set of esterified second fractions;

and/or

10 (2) performing a combined extraction/esterification technique comprising the steps of

- (a) adding to each said reserved portion of biological material, a C5-C8 aliphatic solvent, to form a diluted portion,
- (b) adding to, and mixing with, said diluted portion a C1-C3 alcohol and a corresponding, metal C1-C3 alkoxide, to form an esterified mixture, and
- 15 (c) adding to, and mixing with, said esterified mixture, water
- (d) allowing said aliphatic solvent to coalesce to form a second organic phase, distinct from the remainder of said mixture, and
- (e) separating said second organic phase from said remainder,
- thereby producing an esterified second fraction, each said esterified second fraction being a member in said set of esterified second fractions;
- 20

(H) optionally splitting each said second aqueous phase into a first portion and a second portion;

(I) preparing a set of silylated third fractions by performing

(1) a reconstitution technique comprising the steps of

(a) evaporating to dryness,

(i) each said first aqueous phase, or each said first portion thereof, in the event step (G)(1) is not performed, or

(ii) each said second aqueous phase, or each said first portion thereof, in the event step (G)(1) is performed,

thereby producing a first aqueous phase residue, and

(b) reconstituting said first aqueous phase by adding to, and mixing with, said first aqueous phase residue, a basic nitrogenous organic solvent to produce a reconstituted first aqueous phase; and

(2) a derivatization technique comprising adding to, and reacting with, said reconstituted first aqueous phase, hydroxylamine to form a derivatized first aqueous phase; and

(3) a silylating technique on each said derivatized first aqueous phase,

thereby producing a silylated third fraction, each said silylated third fraction being a member in the set of silylated third fractions;

40 (J) optionally preparing a set of fourth fractions by

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(1) evaporating to dryness:

(a) each said second portion of said original extract; or

(b) either one of

(i) each said second portion of said first aqueous phase, in the event step (G)(1) is not performed, or

(ii) each said second portion of said second aqueous phase, in the event step (G)(1) is performed; or

(c) both (a) and (b),

thereby producing at least one second aqueous phase residue, and

10 (2) reconstituting each said second aqueous phase residue by adding to, and mixing with, said second aqueous phase residue, an aqueous liquid to produce a fourth fraction, each said fourth fraction being a member in the set of fourth fractions;

15 (K) analyzing each of said first fractions, alkylated second fractions, silylated third fractions, and optionally said fourth fractions, to produce at least one set of subject chromatograms and at least one set of control chromatograms, each set representing an analysis of one sample of biological material; and

20 (L) comparing said subject chromatograms with said control chromatograms to identify outlier peaks representing chemically related differences between said subject biological material and said control biological material.

10. The method according to Claim 9, further comprising, after step (L), the step:

(M) determining the chemical identity of at least one outlier peak.

25 11. The method according to Claim 9, wherein in step (C), said fluid extractant comprises

(1) a mixture of KOH and an aqueous solution of about 10-90% isopropanol, or

(2) an aqueous solution of about 10-90% isopropanol.

30 12. The method according to Claim 11, wherein said aqueous solution is about 25-75% isopropanol.

13. The method according to Claim 12, wherein said aqueous solution is about 50% isopropanol.

35 14. The method according to Claim 13, wherein said aqueous solution is about 70% isopropanol.

15. The method according to Claim 11, wherein said mixture comprises about 0.01-0.5N KOH.

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sub 2  
16. The method according to Claim 15, wherein said mixture comprises about 0.1N KOH.

17. (Cancelled).

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18. The method according to any one of Claims 1-8 and 17 wherein, in step (a)(iii), said fluid extractant is aqueous isopropanol or is an aqueous isopropanol-KOH mixture.